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van der Westen, Rebecca ; Hosta-Rigau, Leticia ; Sutherland, Duncan S ; Goldie, Kenneth N ;  
Albericio, Fernando ; Postma, Almar ; Städler, Brigitte

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# Myoblast Cell Interaction with Polydopamine Coated Liposomes

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**Abstract** Liposomes are widely used, from biosensing to drug delivery. Their coating with polymers for stability and functionalization purposes further broadens their set of relevant properties. Poly(dopamine) (PDA), a eumelanin-like material deposited via the “self”-oxidative polymerization of dopamine at mildly basic pH, has attracted considerable interest in the past few years due to its simplicity, flexibility yet fascinating properties. Herein, we characterize the coating of different types of liposomes with PDA depending

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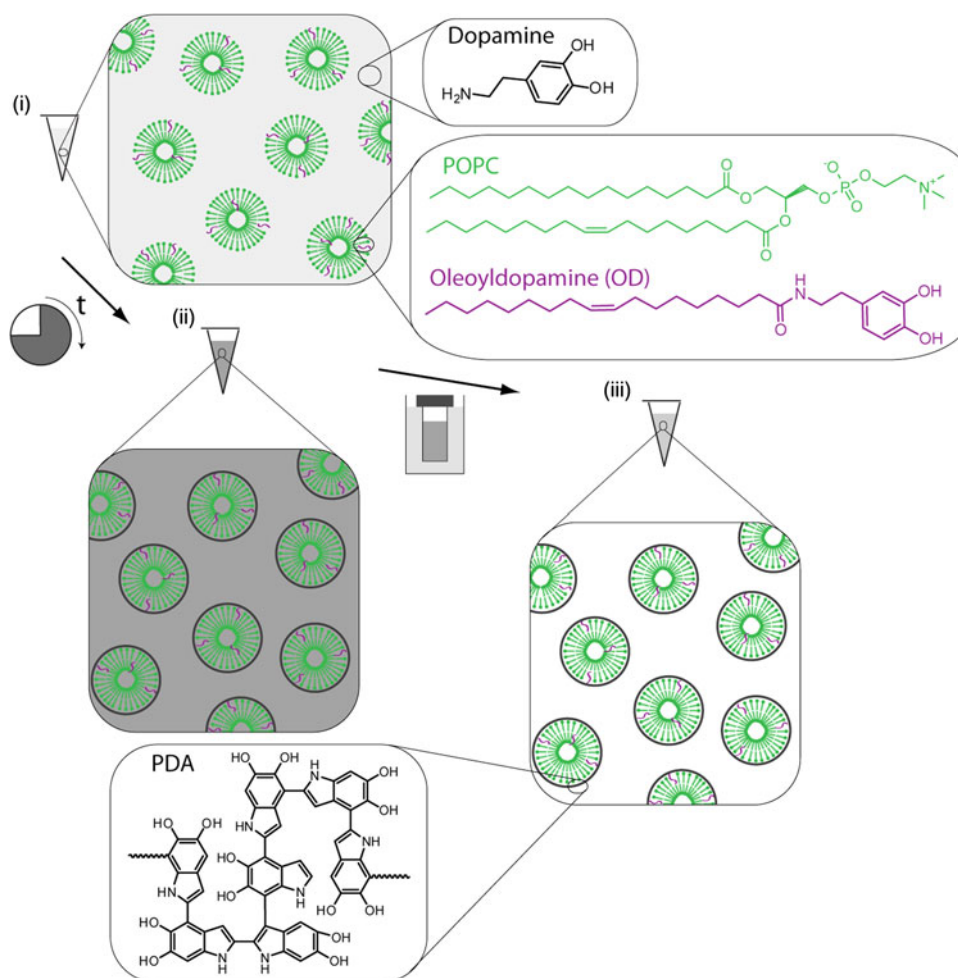
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## 1 Introduction

Liposomes are among the most prominent objects employed in bionanotechnology. In addition to their application as drug delivery vehicles [1, 2], they are also employed in biosensing as labels [3] or as carriers for membrane proteins [4]. Further, they have recently been considered as sub-compartments in polymer capsules towards therapeutic cell mimicry [5–7] or as potential drug deposits embedded in polymer films on surfaces [8, 9].

The combination of polymers with liposomes is interesting in many aspects; beneficial properties from both materials are preserved, while shortcomings can be overcome. Coating liposomes with poly(ethylene glycol) (PEG) using PEGylated lipids is the typical and to date the most successful way to increase the circulation time [10, 11] when liposomes are used in drug delivery. Alternative coatings such as mucoadhesive polymers, e.g. chitosan, have been considered to improve the mucoadhesive properties [12] or modifications based on the sequential deposition of interacting polymers onto liposomes [13]. Although improved properties in terms of stability and cargo retention have been observed in the latter case, the separation of the free polymer

**Fig. 1** Schematic illustration of the PDA coating of liposomes containing OD in the lipid bilayer. A dopamine solution is mixed with a liposome solution (i) and left to react (ii). The sample is then dialyzed yielding a solution of PDA coated liposomes (iii)



during the assembly process is a limiting step. Alternatively, Ali et al. [14] proposed a reversible addition-fragmentation chain transfer (RAFT)-based approach by initially depositing a short polyelectrolyte onto liposomes. The polymer shell thickness was increased by taking advantage of the “living” RAFT moieties which allowed for the further polymer chain extension in the presence of monomers. Once initiated, the polymer shell is “self”-deposited, making this approach potentially widely applicable. On the other hand, the success of the coating relies on the synthesis and deposition of the first polyelectrolyte.

Polydopamine (PDA) [15], a dopamine-derived synthetic polymer, has recently attracted considerable interest as a flexible coating toward biomedical applications [16]. PDA is deposited via a “self”-oxidative process at slightly basic pH on virtually any substrate, independent of material and shape. Many fundamental aspects of PDA have yet to be discovered and characterized, but its photoprotective or electrical properties and options for straight-forward post-functionalization using amines or thiols are making this material highly attractive. Thus, PDA coatings have been considered in a variety of different applications, ranging

from controlling cell/surface interactions e.g. rendering hydrophobic surface cell adhesive [17] to building blocks in biosensing platforms e.g. as matrix polymer for molecular imprinting [18], and for life cell encapsulation [19]. PDA capsules have been considered using particles [20–23] or oil emulsion droplets [24] as templates. Although cargo loading in PDA capsules has been shown, no impact on the cell viability due to the presence of the cargo has been reported and only the absence of inherent cytotoxicity has been demonstrated. Also, the requirement to remove the core potentially limits these approaches. We recently considered PDA coatings for surface-mediated drug delivery by embedding liposomes into the polymer film and demonstrated the uptake of fluorescent lipids from the surface [8]. In this context, we employed X-ray photo spectroscopy to ensure the PDA formation on the liposome coated surfaces, but no further detailed characterization of PDA/liposome interactions has yet been performed.

Herein, we report the coating of liposomes with PDA (Fig. 1) and assess the interaction of myoblast cells with these assemblies as a first step toward their application in drug delivery. In particular we (i) synthesize oleoyldopamine (OD),

(ii) monitor the PDA growth on positively, zwitterionic and negatively charged liposomes with and without OD in their lipid membrane, and (iii) characterize the uptake efficiency of exemplified PDA coated and uncoated liposomes by myoblast cells as well as (iv) the cell cytotoxicity of empty and thiocoraline (TC) loaded PDA coated and uncoated assemblies.

This report is the first detailed characterization of liposome/PDA interaction toward the potential application as drug delivery vehicles.

## 2 Experimental

### 2.1 Materials

Sodium chloride (NaCl), tris(hydroxymethyl)aminomethane (TRIS) buffer, dimethyl sulfoxide (DMSO), dopamine hydrochloride (dopamine), phosphate buffered saline (PBS), and chloroform were purchased from Sigma-Aldrich. Zwitterionic lipids 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC, 25 mg/mL), negatively charged lipids 1-palmitoyl-2-oleyl-*sn*-glycero-3-phospho-L-serine (sodium salt) (POPS, 25 mg/mL), positively charged lipids 1-palmitoyl-2-oleoyl-*sn*-glycero-3-ethylphosphocholine (POEPC, 25 mg/mL), and fluorescent lipids 1-oleoyl-2-[6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoyl]-*sn*-glycero-3-phosphocholine (NBD-PC) dissolved in chloroform were obtained from Avanti Polar Lipids, USA. Thiocoraline (TC) was isolated and purified by PharmaMar, S.A. (Colmenar Viejo, Madrid, Spain).

Two types of buffers were used throughout all of the liposome coating experiments: TRIS<sup>1</sup> buffer consisting of 10 mM TRIS (pH 8.5) and TRIS<sup>2</sup> buffer consisting of 10 mM TRIS and 150 mM NaCl (pH 7.4). All water used in these experiments was tapped from the Millipore water system (Milli-Q gradient A 10 system, resistance 18 MΩ cm, TOC < 4 ppb, Millipore Corporation, USA).

### 2.2 Synthesis of Oleoyldopamine

*N*-Oleoyl dopamine, a commercially available compound with biomedical research interest [25–27] was synthesized via the amidation of oleoyl chloride.

Oleoyl chloride was synthesized according to standard acid chloride synthesis via oxalyl chloride obtaining a similar yield as published [28].

Dopamine hydrochloride (1.89 g, 9.97 mmol) was dissolved in dry dimethylformamide (DMF, 25 mL) at room temperature (RT), under argon in a two neck round bottom flask (100 mL). Triethylamine (TEA, 1.51 g, 14.9 mmol) was added dropwise under stirring and then cooled to −20°C in an ethanol/dry ice bath. Oleoyl acid chloride (1.5 g, 4.98 mmol) was dissolved in dry dichloromethane

(DCM) and added dropwise over 1 h to the reaction at −20°C. After addition, the heterogeneous mixture was allowed to come to 0°C for 40 min, and then brought to RT and left over night.

The reaction mixture was diluted with 75 mL DCM and washed 2× with 0.5 M potassium bisulfate (50 mL), water (25 mL), brine (50 mL) and filtered through silica. The material was dried under vacuum to solidify to an off-white waxy solid. This was dissolved in methanol and precipitated with the addition of water (2×) to give white crystals, 1.58 g (88.9% yield), HPLC purity >99%.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.75, s, 1H, OH; 6.81, d, *J* 8.0 Hz, 1H, CH (Ar); 6.75, d, *J* 2.0 Hz, 1H, CH (Ar); 6.56, dd, *J* 8.0, 1.9 Hz, 1H, CH (Ar); 6.07, s, 1H, OH; 5.63, t, *J* 5.6 Hz, 1H, NH; 5.34, m, 2H, =CH; 3.48, m, 2H, CH<sub>2</sub>N; 2.69, t, *J* 7.1 Hz, 2H, CH<sub>2</sub>Ar; 2.15, m, 2H, CH<sub>2</sub>CO; 1.99, m, 4H, CH<sub>2</sub>CH=; 1.58, m, 2H, CH<sub>2</sub>CH<sub>2</sub>CO; 1.26, m, 20H, CH<sub>2</sub>; 0.88, t, *J* 6.8 Hz, 3H, CH<sub>3</sub>.

### 2.3 Liposome Formation

Unilamellar liposomes were prepared by evaporation of the chloroform of the lipid solutions under vacuum for 1 h. Zwitterionic liposomes consisted of 2.5 mg POPC lipids with 0 wt% OD (L<sub>OD,0</sub><sup>zw</sup>), 0.5 wt% OD (L<sub>OD,0.5</sub><sup>zw</sup>), or 2.5 wt% OD (L<sub>OD,2.5</sub><sup>zw</sup>). Negatively charged liposomes were made from 0.5 mg POPS and 2 mg POPC lipids with 0 wt% OD (L<sub>OD,0</sub><sup>−</sup>), 0.5 wt% OD (L<sub>OD,0.5</sub><sup>−</sup>), or 2.5 wt% OD (L<sub>OD,2.5</sub><sup>−</sup>). Positively charged liposomes consisted of 0.5 mg POEPC and 2 mg POPC lipids with 0 wt% OD (L<sub>OD,0</sub><sup>+</sup>), 0.5 wt% OD (L<sub>OD,0.5</sub><sup>+</sup>), or 2.5 wt% OD (L<sub>OD,2.5</sub><sup>+</sup>). For fluorescently labelled liposomes (NBDL), 0.5 wt% of NBD-PC was added to the lipid solution. Thiocoraline (TC) loaded liposomes (TC<sub>L</sub>) were assembled by adding 100 μL (0.1 mg/mL) TC in chloroform to the lipid mixture prior to drying. This TC concentration was chosen to ensure the highest amount of incorporated cargo into the lipid membrane of the liposomes based on our previous reports [29, 30]. Thus, increasing TC concentration above 0.1 mg/mL does not results in an increase in the amount of liposome-loaded TC. The dried lipid film was rehydrated with 1 mL TRIS<sup>1</sup> buffer and the solution was extruded through 100 nm filters (11 times).

### 2.4 PDA Coating of Liposomes

The nine types of liposomes were coated with PDA according to the following protocol. 10 mg/mL dopamine stock solution in TRIS<sup>1</sup> buffer was prepared and used as is or diluted to two different dopamine concentrations (2 and 5 mg/mL) using TRIS<sup>1</sup> buffer. Typically, 125 μL of liposome solution was mixed with 125 μL dopamine solution leading to dopamine concentrations of D<sub>1</sub> = 1 mg/mL,

$D_{2.5} = 2.5$  mg/mL and  $D_5 = 5$  mg/mL during coating. Three control samples of 125  $\mu$ L dopamine (2, 5 and 10 mg/mL) mixed with 125  $\mu$ L TRIS<sup>1</sup> buffer were run in parallel. The samples were permanently shaken during the coating process. The size and polydispersity (PD) of the samples was determined at different time points by diluting 30–50  $\mu$ L sample solution in 700  $\mu$ L TRIS<sup>1</sup> prior to measuring in a dynamic light scattering (DLS) instrument (Zetasizer nano, Malvern Instruments) using a material refractive index of 1.590 and a dispersant (water at 25°C) refractive index of 1.330. Within this paper, samples with a PD > 0.4 were considered aggregated and were discarded. As the final step, the PDA coating process was stopped and the free dopamine and small PDA aggregates in solution were removed by diluting the sample 1:1 v/v with TRIS<sup>2</sup> buffer followed by dialysis against TRIS<sup>2</sup> buffer overnight. This step also adjusted the buffer solution to physiological conditions.

The long-term stability of  $L_{OD\_0}^+$  and  $L_{OD\_0D_{2.5}}^+$  was assessed by storing them in either TRIS<sup>2</sup> buffer or TRIS<sup>2</sup> buffer supplemented with 2 mg/mL BSA at  $T = 4^\circ\text{C}$ , at room temperature or at  $T = 37^\circ\text{C}$ . Their size and PD was regularly measured by DLS over max 3 weeks.

For the subsequent experiments,  $L_{OD\_0}^+$  (supplemented with NBD-PC or TC if required) using 1 or 2.5 mg/mL dopamine ( $L_{OD\_0D_1}^+$  or  $L_{OD\_0D_{2.5}}^+$ ) and a polymerization time of 75 min was chosen. As a control, samples of  $L_{OD\_0}^+$  without dopamine but with the same dilution steps were also prepared.

## 2.5 Cryo-Transmission Electron Microscopy (cryo-TEM)

Samples were prepared for cryo-TEM by adsorbing 4  $\mu$ L of the liposome suspension onto Quantifoil R3.5/1 holey carbon film mounted on 300 mesh copper grids (Quantifoil Micro Tools GmbH, Jena, Germany). Prior to adsorption, the grid was rendered hydrophilic by glow discharge in a reduced atmosphere of air for 10 s. The specimen was applied and after 1 min incubation on the surface, the grid was blotted and quick-frozen in liquid ethane using a Vitrobot automated plunging device (FEI Company, Eindhoven, The Netherlands).

The frozen grids were transferred to liquid nitrogen before loading into a Gatan 626 cryo-holder (Gatan, Pleasanton, CA, USA). The cryo-holder was then inserted into the stage of a Philips CM200 FEG TEM (FEI company) operated at 200 kV. Imaging was performed at cryogenic temperatures (approx.  $-170^\circ\text{C}$ ) in low-dose, bright-field mode. Electron micrographs were recorded digitally on a TVIPS 4 k  $\times$  4 k CMOS Camera (TVIPS GmbH, Gauting Germany) at given defocus values of  $-2.5$   $\mu\text{m}$ .

## 2.6 Cell Experiments

The C2C12 mouse myoblast cell line was used for all the experiments. The cells were cultured as monolayers in 75 cm<sup>2</sup> culture flasks in medium (Dulbecco's modified Eagle's Medium with Glutamax (DMEM) supplemented with 10% fetal bovine serum, 50 U/mL penicillin, 50  $\mu$ g/mL streptomycin and 1 mM sodium pyruvate, all from Invitrogen) at 37°C and 5% CO<sub>2</sub>. The cells were seeded into 96-well plates at a density of 7,500 cells/well in 200  $\mu$ L medium and allowed to attach for 20 h at 37°C and 5% CO<sub>2</sub> prior to the uptake and cell viability experiments.

### 2.6.1 Uptake Experiments

8 or 32  $\mu$ L of  $^{NBD}L_{OD\_0}^+$  or  $^{NBD}L_{OD\_0D_{2.5}}^+$  solution was added per well and the cells were exposed to this liposome-containing media for different time points (3, 6 or 24 h). Then, the cells were washed 2 $\times$  in 300  $\mu$ L PBS, trypsinized and re-suspended in 200  $\mu$ L PBS for analysis by flow cytometry. A C6 Flow Cytometer (Accuri Cytometers Inc) using an excitation wavelength of  $\lambda = 488$  nm was employed to measure the fluorescence intensity of the cells upon their association with these assemblies. At least 3,000 cells were analyzed. The experiments were performed in triplicates and three independent repeats.

### 2.6.2 Cell Viability

The amount of encapsulated TC was quantified by fluorescence spectrophotometry. The different samples were excited at a wavelength of 365 nm and the fluorescence intensity was recorded at an emission wavelength of 547 nm. The concentration of TC was determined by correlation with a calibration curve (Figure S1, Supporting Information). The experiments were carried out using a multi plate reader (PerkinElmer). The averaged encapsulated TC in the  $^{TC}L_{OD\_0}^+$  stock solution was  $7.5 \pm 1.8$   $\mu\text{g/mL}$ . We further assumed that the PDA coating does not affect the TC retention.  $^{TC}L_{OD\_0}^+$  or  $^{TC}L_{OD\_0D_1}^+$  solutions with different concentrations (between 0 and 1.2  $\mu\text{g/mL}$ ) of TC were added per well and incubated with the cells for 24 h. The cell viability was assessed using the Cell Counting Kit-8 (Dojindo) by adding 20  $\mu$ L assay solution per well and an incubation time of 2 h prior to the absorbance read out using a multi plate reader (PerkinElmer). The results were background corrected by subtracting the absorbance reading for media only and normalized to untreated cells. The experiments were performed in triplicates and three independent repeats.



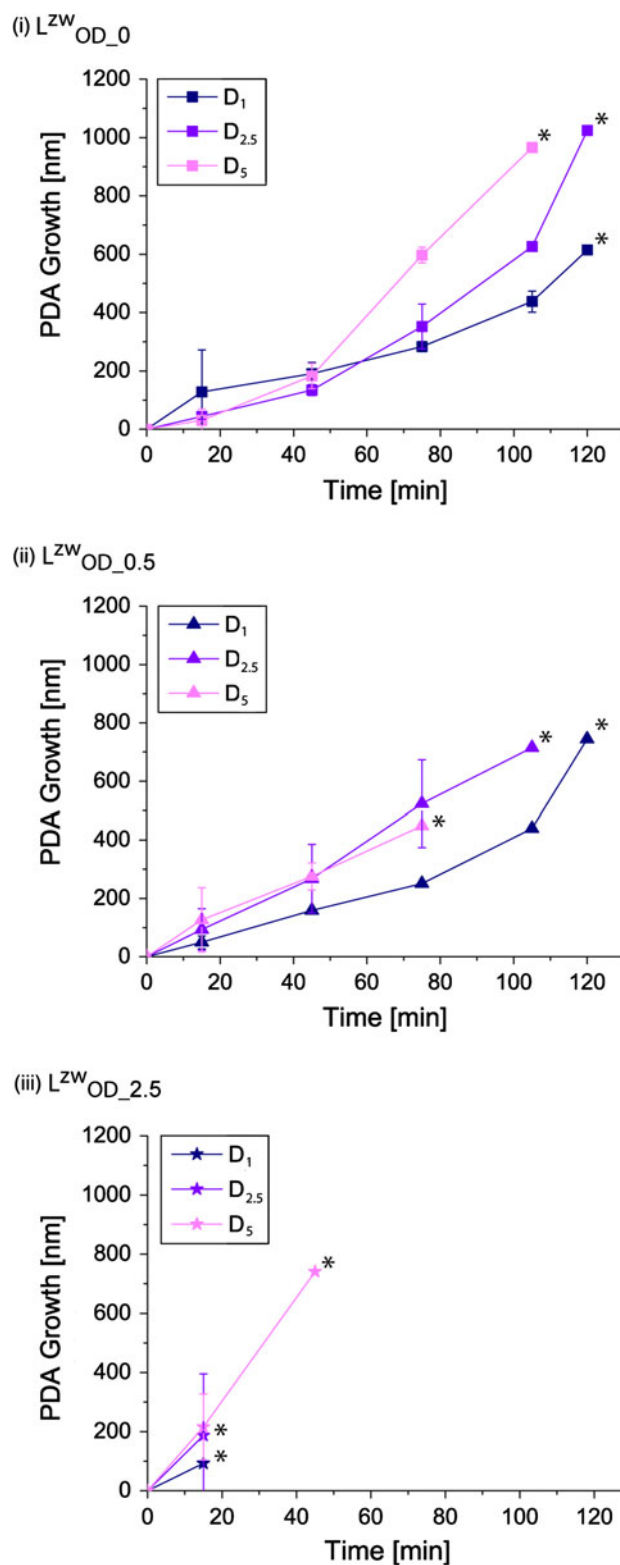
### 3 Results and Discussion

#### 3.1 PDA Coating of Liposomes

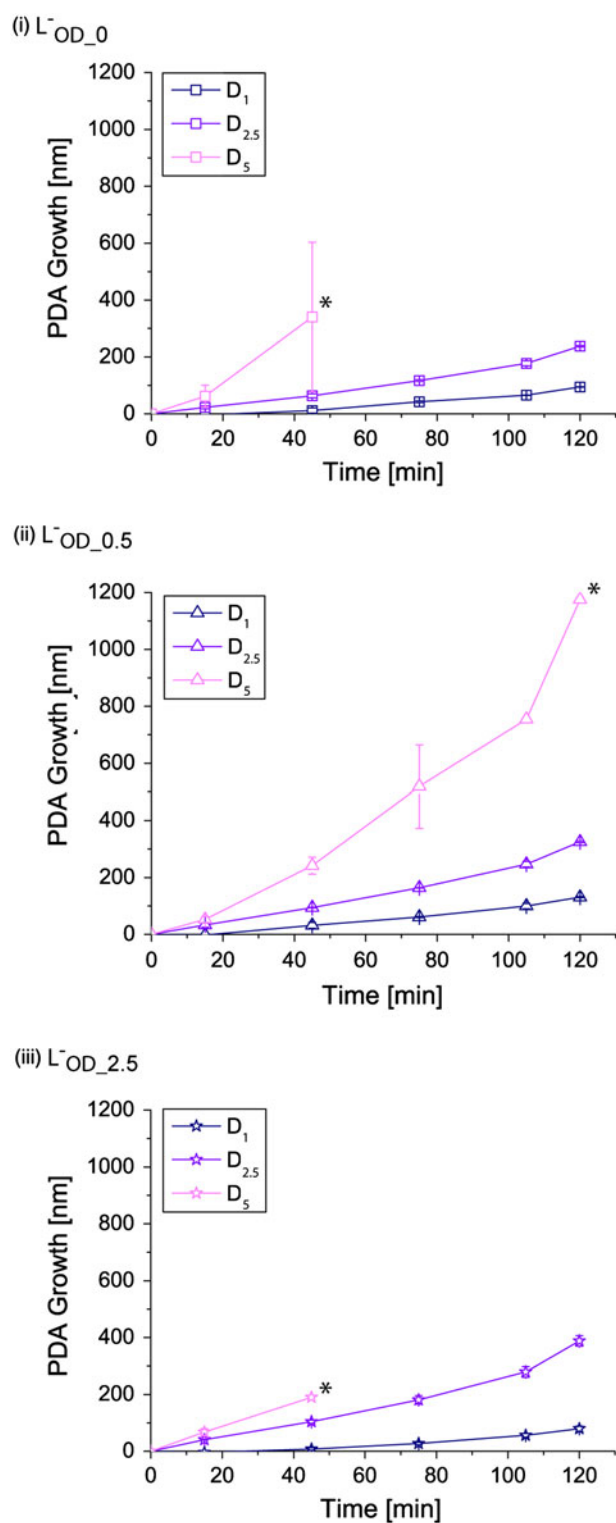
With the aim to characterize the effect of the lipid composition and the presence of OD in the lipid membrane on the PDA growth rate, zwitterionic, negatively, and positively charged liposomes with different amounts of incorporated OD were exposed to dopamine solutions with different concentration. OD was embedded within the membrane with the goal to facilitate the PDA deposition i.e. to serve as anchor since it will copolymerize with the dopamine/PDA. The change in liposome diameter and PD over time was monitored using DLS (Fig. 1i, ii). We would like to note that the quantitative change in diameter could be misleading due to the deposited PDA around the liposomes. This black coating is expected to absorb the laser light and thus could affect the outcome of the measurement. However, qualitatively it is nonetheless possible to compare the PDA growth rates between the different samples. Figure 2 summarizes the result for the zwitterionic liposomes. In general, both the presence of OD and the different concentrations of dopamine affected the PDA growth rate. Increasing amounts of OD led to faster PDA growth rates for the same dopamine concentration. On the other hand, increasing concentration of dopamine did not affect the PDA growth rate, but sample aggregation was observed after shorter times. After a max of 120 min, all the tested samples had a PD > 0.4 and were considered aggregated [marked by an asterisk (\*) in Fig. 2]. While the presence of OD could be an advantage in a different context e.g. when the liposomes are adsorbed to the surface prior to the PDA coating, here, for the PDA coating of zwitterionic liposomes in solution, a too high amount of OD is not beneficial.

On the other hand, the presence of OD in negatively charged liposomes was neither affecting the PDA growth rate nor the aggregation behavior of the samples (Fig. 3). However, in this case increasing amounts of dopamine yielded faster growth of the liposome's diameter. Only the highest dopamine concentration tested caused aggregation of the samples after a max of 120 min. All the other combinations preserved a PD < 0.4.

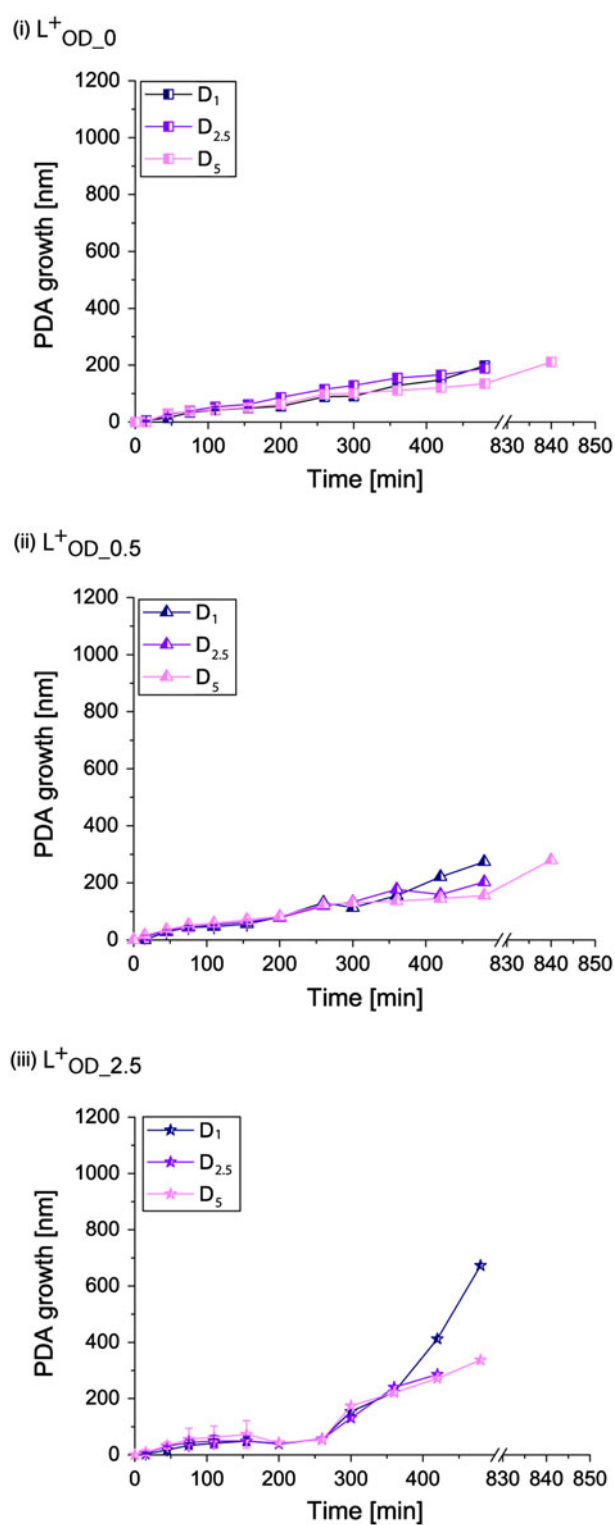
The coating of the third type of tested liposomes, the positively charged ones, was neither affected by OD nor by increasing amounts of dopamine, but exhibited a slow but stable PDA growth during the max monitored time of 800 min (Fig. 4). These observations suggest that the electrostatic interaction of the dopamine with the liposomes is dominating over the presence of OD. Further, DLS measures the hydrodynamic radius and therefore, the properties i.e. stiffness of the deposited PDA film, could vary depending on the type of liposomes used as template.



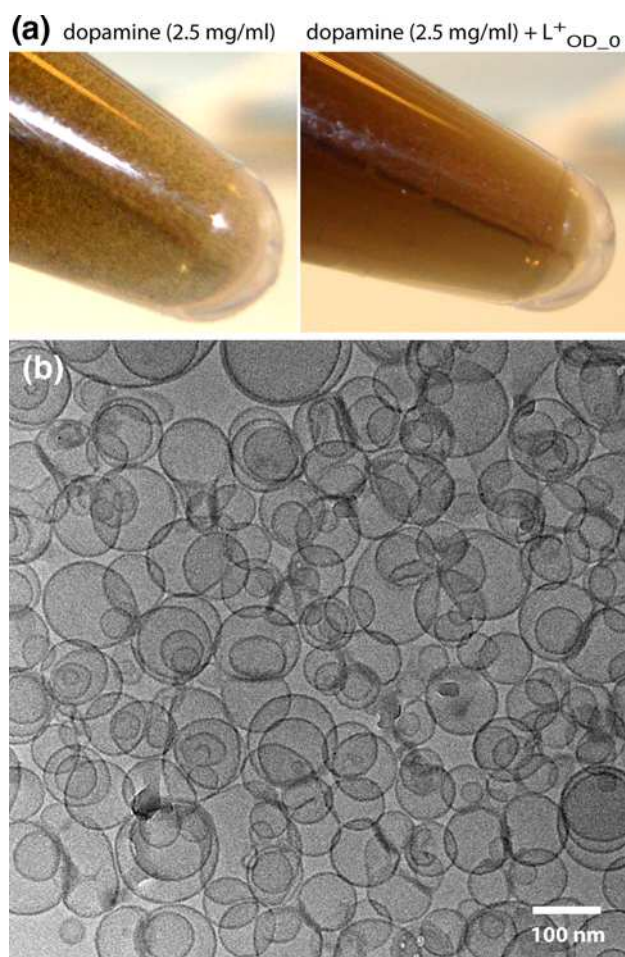
**Fig. 2** Time-course of the normalized increase in diameter for the PDA assembly on zwitterionic liposomes with three different amounts of OD [(i) 0, (ii) 0.5 and (iii) 2.5 wt%] using three different concentrations of dopamine (1, 2.5 and 5 mg/mL). All the samples had a PD > 0.4 after 120 min as indicated by asterisks



**Fig. 3** Time-course of the normalized increase in diameter for the PDA assembly on negatively charged liposomes with three different amounts of OD [(i) 0, (ii) 0.5 and (iii) 2.5 wt%] using three different concentrations of dopamine (1, 2.5 and 5 mg/mL). The asterisks indicates samples with a  $PD > 0.4$



**Fig. 4** Time-course of the normalized increase in diameter for the PDA assembly on positively charged liposomes with three different amounts of OD [(i) 0, (ii) 0.5 and (iii) 2.5 wt%] using three different concentrations of dopamine (1, 2.5 and 5 mg/mL)



**Fig. 5** **a** Photographs of solutions containing 2.5 mg/mL dopamine in TRIS<sup>1</sup> (left) or 2.5 mg/mL dopamine and L<sup>+</sup><sub>OD\_0</sub> in TRIS<sup>1</sup> (right) after 75 min, showing a flocculated (left) and a homogenous (right) dark brown solution, respectively. **b** Cryo-TEM image of L<sup>+</sup><sub>OD\_0</sub>D<sub>1</sub> showing structurally intact liposomes

However, the qualitative comparison between the different samples is still valid. A detailed investigation of this aspect is beyond the scope of this initial paper and will be part of a subsequent publication. Although positively charged liposomes had the slowest PDA growth rate, the absence of aggregation makes them the most suitable candidates for the purpose outlined in this paper.

Interestingly, dopamine solutions containing no liposomes started to show aggregated PDA after 15–30 min with sizes of 200–400 nm (PD ~ 0.3) which increased in size such that they flocculated out of solution with several micron sized aggregates (PD = 1) after 75 min (Fig. 5a, left). On the other hand, when the liposomes e.g. L<sup>+</sup><sub>OD\_0</sub> were present in the dopamine solution of the same concentration, the solutions turned a homogenous dark brown (Fig. 5a, right). In general, this has been observed independent of the type of liposomes used, but the effect was more pronounced for the charged liposomes without

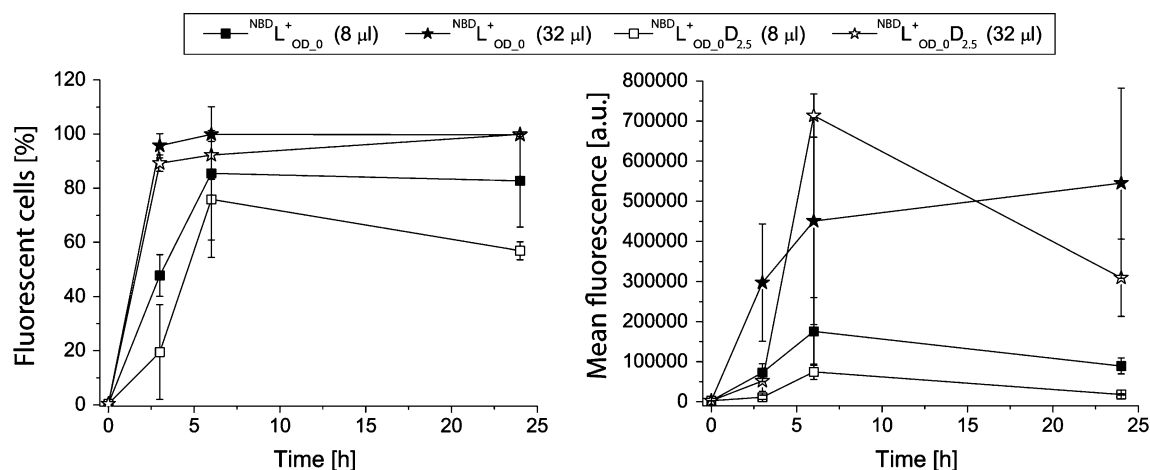
OD. While we do not fully understand the reasons, we speculate that the liposomes were a preferred deposition site over the PDA aggregates in solution themselves. This fact allows for the purification of the coated liposomes by dialysis only, without the need for additional steps such as centrifugation.

While we are not aiming at assembling PDA capsules, i.e. removal of the liposomes, but to equip the liposomes with a stabilizing adhesive layer, L<sup>+</sup><sub>OD\_0</sub> with 1 or 2.5 mg/mL dopamine (L<sup>+</sup><sub>OD\_0</sub>D<sub>1</sub> and L<sup>+</sup><sub>OD\_0</sub>D<sub>2.5</sub>) with a polymerization time of 75 min was chosen for all the subsequent experiments. The subsequent purification step via dialysis against TRIS<sup>2</sup> caused the PDA coating to keep growing and yielded stable, PDA coated liposomes as shown in a representative cryo-TEM image of L<sup>+</sup><sub>OD\_0</sub>D<sub>1</sub> in Fig. 5b. Cryo-TEM images of L<sup>+</sup><sub>OD\_0</sub>D<sub>2.5</sub> looked very similar (Results not shown). It demonstrates that the liposomes remained intact without distortion or visible damage to the membrane due to the PDA coating. However, it also shows that the diameter assessed by DLS was overestimated, likely due to the effect of the light-absorbing coating on the liposomes on the DLS measurements. Further, no large PDA particles (<100 nm) were observed confirming that dialysis as a purification step is sufficient. The PDA coated liposomes were found to be stable for at least 2 weeks at 4°C, room temperature, and 37°C in TRIS<sup>2</sup> buffer with and without BSA (results not shown).

### 3.2 Interaction of PDA Coated Liposomes with Myoblast Cells

Since we aim to use the coated liposomes as drug delivery vehicles or as subunits in larger assemblies, we characterized the uptake efficiency by myoblast of <sup>NBD</sup>L<sup>+</sup><sub>OD\_0</sub>D<sub>2.5</sub> in comparison to uncoated <sup>NBD</sup>L<sup>+</sup><sub>OD\_0</sub>. All the diameters mentioned were measured by DLS, and as pointed out previously the dark coating was likely to affect the read out, allowing only qualitative comparing between the different samples. In order to understand how the L<sup>+</sup><sub>OD\_0</sub>D<sub>2.5</sub> association with the myoblast cells evolves over time, myoblast cells were incubated with two different concentrations of <sup>NBD</sup>L<sup>+</sup><sub>OD\_0</sub>D<sub>2.5</sub> or <sup>NBD</sup>L<sup>+</sup><sub>OD\_0</sub> for 3, 6 or 24 h followed by the monitoring of the fluorescence intensity of the cells by flow cytometry (Fig. 6). The uptake efficiency showed that after 6 h 100 and 80% of the cells in the population had liposomes associated with them for the higher and lower tested concentration, respectively and remained on a similar level for the tested 24 h (Fig. 6, left). In parallel, the mean fluorescence of the cells, as a more quantitative measure of the amount of liposomes associated per cell, showed that the 4× higher concentration of L<sup>+</sup><sub>OD\_0</sub>D<sub>2.5</sub> or L<sup>+</sup><sub>OD\_0</sub> resulted in ~4× higher fluorescence intensity of the cells (Fig. 6, right). Further, although the



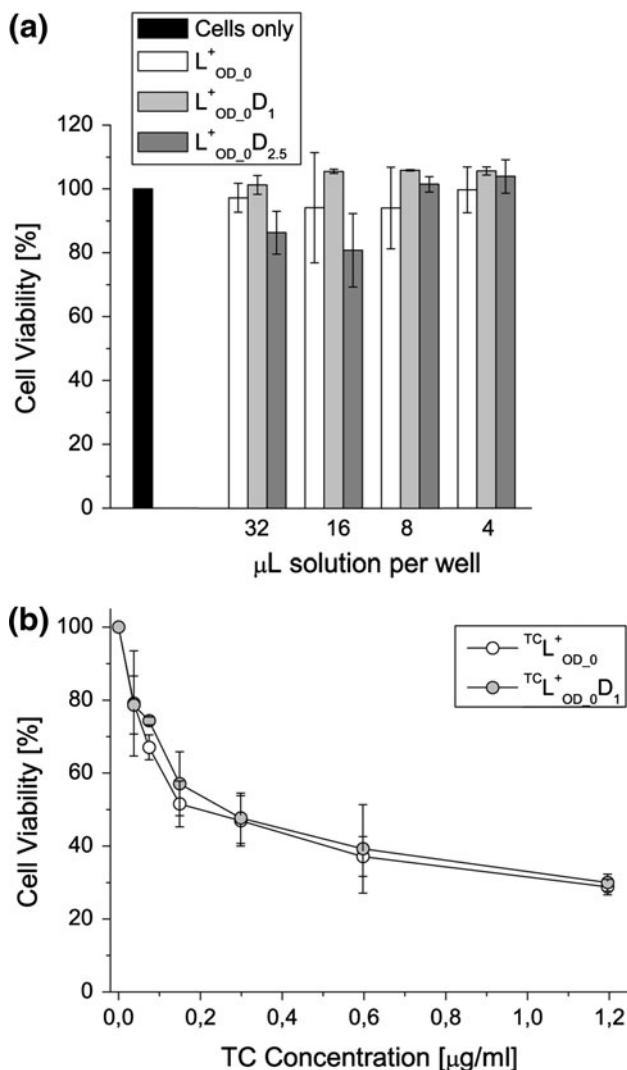


**Fig. 6** Uptake efficiency (*left*) and mean fluorescence (*right*) of myoblast cells incubated with  $NBD-L_{OD-0}^+$  or  $NBD-L_{OD-0D2.5}^+$  (8 and 32  $\mu$ L per well) for different time points as measured by flow cytometry

fluorescence intensity of the  $NBD-L_{OD-0D2.5}^+$  and  $NBD-L_{OD-0}^+$  solutions were within  $\pm 10\%$  as measured by fluorescence spectrophotometry, the fluorescence intensity of the myoblasts was doubled when exposed to  $L_{OD-0D2.5}^+$  as compared to  $L_{OD-0}^+$ . This suggests that the PDA coating might promote their uptake by/association with the cells. In addition, for most cases, the fluorescence intensity of the cells dropped between 6 and 24 h, suggesting that the cells either processed the fluorescent lipids or divided, and by doing so were reducing the fluorescence intensity per cell. The only exception was the higher concentration of  $NBD-L_{OD-0}^+$ , probably due to the high concentration of liposomes present but slower uptake/association kinetics especially compared to  $L_{OD-0D2.5}^+$ , again possibly explained by the different outmost layer, PDA versus phospholipids, and this aspect is currently part of a more detailed investigation.

Understanding if the coated liposomes have inherent cytotoxic effects is of crucial importance when these assemblies are considered for biomedical applications i.e. as drug delivery vehicles. To this end, we exposed different concentrations of  $L_{OD-0D2.5}^+$ ,  $L_{OD-0D1}^+$ , or  $L_{OD-0}^+$  to myoblasts for 24 h and assessed the cell viability. There was no significant effect on the viability of the cells observed for both tested coating thicknesses, confirming the insignificant cytotoxicity of the PDA coating (Fig. 7a). This result is in good agreement with the previously reported negligible cytotoxicity of PDA capsules to LIM1215 cells [20].

In a next step, we employed the small cytotoxic hydrophobic depsipeptide thiocoraline (TC), [31] in order to investigate in a feasibility study, if the PDA coating affects the potential cargo delivery. First, the entrapped TC did not affect the PDA coating of the liposomes, yielding  $TC-L_{OD-0D1}^+$  with an average diameter of  $393 \pm 45$  nm and a PD of  $0.18 \pm 0.02$ , similar to what was measured for  $L_{OD-0D1}^+$ . A comparable dose dependent viability of the



**Fig. 7** **a** Normalized cell viability of myoblast cells measured after 24 h exposure to different concentrations of  $L_{OD-0}^+$ ,  $L_{OD-0D1}^+$  and  $L_{OD-0D2.5}^+$ . **b** Normalized cell viability depending on the TC concentration in  $TC-L_{OD-0}^+$  and  $TC-L_{OD-0D1}^+$

cells was observed when TC was entrapped within the lipid bilayer of the liposomes for  $TC_{L_{OD_0}^+}$  and  $TC_{L_{OD_0}^+D_1}$  (Fig. 7b), suggesting that the presence of PDA and the used coating thickness did not significantly hinder the activity and release of TC, a compound which acts by unwinding negatively supercoiled double-stranded DNA and binding to DNA by bisintercalation [32].

#### 4 Summary and Conclusions

We report the coating of liposomes with PDA and assess the interaction of these assemblies with myoblast cells. The PDA growth rate depended on the charge of the used liposomes, the dopamine concentration and the presence of OD within the lipid bilayer. The coated liposomes were found to be stable in physiological conditions for at least 2 weeks and associated with myoblast cells in a similar amount as for the uncoated liposomes. Further, the PDA coating did not affect the viability of these cells unless the cytotoxic depsipeptide TC was entrapped within the lipid bilayer. Taken together, this first report on coating liposomes with PDA demonstrates their potential as drug delivery vehicle.

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**Conflict of interest** The authors declare that they have no conflict of interest.

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